

Response of benthic assemblages to multiple stressors: comparative effects of nutrient enrichment and physical disturbance

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Abstract

Stressors to ecological communities often overlap in time and space and may have additive, synergistic or antagonistic effects. Nutrient enrichment and physical disturbance are two commonly co-occurring stressors to estuarine ecosystems, but their combined effects have mainly been investigated in mesocosm experiments of unknown relevance to field scenarios. Here, the interacting effects of these two stressors were examined at two field locations – Botany Bay and Lane Cove, New South Wales, Australia – using a fully orthogonal manipulative experiment. All possible combinations of zero, low and high intensities of nutrient enrichment and physical disturbance on macrofaunal and microphytobenthic communities were examined. Effects of stressors were generally site-specific and additive, differing in terms of magnitude of effects,

although some idiosyncratic interactive effects were seen on selected species. Where effects of stressors were observed, nutrient enrichment generally increased microphytobenthic biomass and altered the macrofaunal community structure while physical disturbance produced limited impacts. The divergent results of this and previous mesocosm experiments, which found primarily interactive effects of the stressors, highlights the importance of undertaking field experiments that offer a greater element of realism. Furthermore, this study, in finding differing responses to stressors at the two sites, highlights the importance of environmental context in mediating effects.

Key words:

Multiple Stressors; Context dependence; Nutrient Enrichment; Disturbance; Macrobenthos, Microphytobenthos, Field Experiments

Introduction

How organisms respond to naturally occurring and anthropogenic stressors has been a subject that has dominated the marine ecological literature for many decades (Dayton 1971, Connell 1978, Paine & Levin 1981, Menge & Sutherland 1987, Hall 1994, Crain et al. 2008). Early studies considered the effects of stressors independently from one another and on individual species, yet stressors to natural ecological systems rarely occur singularly (Crain et al. 2008, Halpern et al. 2008) and species rarely occur alone. Effects of multiple stressors cannot easily be predicted from the effects of singular stressors because, when combined, they are rarely neutral but have additive, subtractive or synergistic effects (Crain et al. 2008, Darling & Côté 2008, Bijma et al. 2013). Yet many studies continue to examine the effects of stressors individually and on single

species or small sub groups of taxa (Crain et al. 2008). As coastal development and climate change continue to increase the frequency and intensity of stressors (Halpern et al. 2008, Bijma et al. 2013) there is need to understand the combined effects of stressors, so as to develop appropriate policy and management strategies to manage and perhaps minimise their impact.

Of the studies that have considered the effects of multiple stressors on organisms, many have been conducted in the controlled environment of experimental mesocosms, with species isolated from their habitats and communities (Hicks et al. 2011, Godbold et al. 2011). Although such studies allow the effects of stressors to be examined in the absence of other confounding influences, the extent to which results can be applied to natural systems is unclear because they fail to take into consideration the role of the environmental and biotic context in mediating stressor impacts. For example, organisms can make use of microhabitats to minimise exposure to stressors (Jones & Boulding 1999) and, under natural conditions, ecological interactions such as competition, facilitation and predation may dampen or exacerbate stressor impacts (Christensen et al. 2006, Hicks et al. 2011). *In situ* mesocosms have been used effectively to add an element of realism to assessments of stressor impacts (e.g. Christensen et al. 2006). Nevertheless, while studies such as these offer interesting insights and explore important relationships between changes in ecosystem function and biodiversity under scenarios of environmental change, they do not represent natural conditions and field studies are still needed as part of the experimental framework (for a review of the topic see Crain et al. 2008; Przeslawski et al. 2015 and references therein).

61 Estuarine environments are among the most heavily impacted in the world, with greater than
62 40% of the world's human population living within 100 km of the coast. Among the plethora of
63 stressors affecting these systems on a daily basis, nutrient enrichment and physical disturbances
64 of sediments are often singled out as the most severe (Gray 1997). Leaching of nutrients from the
65 terrestrial environment, atmospheric deposition and decomposition are natural sources of
66 nutrient loading in these environments but these can become exacerbated by human influences
67 (Nixon 1995). For example, the discharge of sewage into aquatic environments, as well as run-off
68 of nutrient-rich fertilisers, cleaning products and animal wastes from the land can enhance
69 delivery of nitrogen and phosphorus to estuarine systems (Nixon 1995, Vitousek et al. 1997a).
70 Where nitrogen and/or phosphorous is a limiting resource, addition of these nutrients has the
71 potential to greatly stimulate primary productivity (Vitousek et al. 1997a, Cloern 2001). Excess
72 nutrients have been linked to eutrophication events worldwide whereby algal blooms are
73 facilitated, ultimately leading to hypoxic and anoxic conditions through over-stimulation of
74 oxygen-consuming microbial decomposition (Vitousek et al. 1997b).

75 High human population densities around estuaries also facilitate many small scale disturbances to
76 estuarine sediments through recreation – e.g. boat wake, anchorage, propeller scarring, coastal
77 walking (Bishop 2005, Rossi et al. 2007) – and as a consequence of livelihood exploitation – e.g.
78 bait digging, fishing, dredging (Wynberg & Branch 1994, Somerfield et al. 1995, Brown & Wilson
79 1997). These physical disturbances have been widely studied and can lead to the disturbance and
80 redistribution of sediments (Hall 1994). Such disturbances are likely to cause physical damage to
81 sediment-dwelling organisms or burrows (Hall & Harding 1997). This physical disturbance has the

potential to alter the distribution, abundance and diversity of macrofaunal assemblages (Fraterrigo & Rusak 2008). At a larger scale, natural events such as storms also cause physical disturbances to sediments although less frequently, offering the potential for recovery of sediment communities between events. These events loosen sediment, making it more susceptible to erosion and further stimulating damaging scour impacts (Yeo & Risk 1979). While sediment grain size can mediate the effect of disturbance on communities (Lindegarth & Hoskin 2001), grain size itself can also become altered by intense disturbance effects (Bishop 2005) thereby facilitating a change in community structure.

The aim of this study was to explore how the stressors, nutrient enrichment and physical disturbance, interact under natural field conditions. Nutrient enrichment and physical disturbances often overlap in time and space. For example, nutrient enrichment influences whole catchments, within which physical disturbances may also occur at smaller scales. Additionally, storm events that flush nutrient pollution into estuarine and coastal areas may also cause physical disturbance (Harris 2014). Despite this, most studies that have examined effects of these stressors on sediment communities have done so independently, and those that have examined interacting effects have done so in small-scale mesocosm experiments (see Widdicombe & Austen 2001, Austen & Widdicombe 2006). According to Huston's (1979) dynamic equilibrium model, that posits that larger disturbances will be required to disrupt competitive dominance when productivity is high, the two stressors are predicted to display non-additive effects. Mesocosm experiments provide support for this hypothesis (Widdicombe & Austen 2001, Austen & Widdicombe 2006). In buckets, experimental manipulations of nutrient enrichment (via addition

of dried and powdered *Ascophyllum*) and physical disturbance (via surface raking) revealed that diversity was lower than expected when low frequencies of physical disturbance were applied in combination with high levels of organic enrichment or vice versa (Widdicombe & Austen 2001, Austen & Widdicombe 2006). Diversity was higher than expected when both disturbance and enrichment were either high or low (Widdicombe & Austen 2001). Despite these results, it is unknown whether these non-additive effects also occur in field systems in which environmental conditions may buffer effects and recruitment of fauna is possible. Here, manipulative field experiments were conducted in two estuaries of eastern Australia to test the hypotheses that (1) the two stressors would display non-additive effects on microphytobenthic and macrofaunal communities, that are not easily predicted from their independent effects and (2) the nature of interactions would vary across field sites that differ in biotic and abiotic conditions.

Materials and Methods

Study site

Field experiments were conducted between May and September 2012 at two locations within Sydney, New South Wales, Australia: Tambourine Bay within Lane Cove River (33°49'39"S 151°09'38"E); and Woollooware Bay within Botany Bay (34°01'11"S, 151°07'46"E). Sites were unvegetated intertidal mud flats, adjacent to mangrove habitat. Each was within 15 km of the estuary mouth, was situated in a highly urbanised catchment, and had sandy-mud sediments. Sites were away from storm-water drains, which transport nutrients into estuaries. Although the sites may be subject to some, minimal, trampling and the occasional boat run-aground, neither was subject to background raking or substantial bait-digging prior to experimental intervention.

124 At an intertidal elevation of ~0.5 m above mean low water springs, 70 square plots (0.5 x 0.5 m in
125 size) were randomly established, and marked by a single post in the upper left corner of the plot.
126 Each plot was separated by approximately 3 metres.

127 **Experimental approaches**

128 The interactive effect of stressors was determined using a fully orthogonal experimental design
129 with two factors, nutrient enrichment and physical disturbance. Each factor had three levels of
130 disturbance intensity: zero, low or high. Seven plots were randomly assigned to each of the
131 resulting nine experimental treatments. The remaining seven plots were procedural controls for
132 the method of nutrient enrichment (see below).

133 Physical disturbance was manipulated by raking sediments (50 cm wide rake) to a depth of 2–4 cm.
134 Raking is one of the most commonly used methods for physically disturbing sediments (e.g. Cowie
135 et al. 2000, Whomersley et al. 2010), and mimics cockle hand raking (Kaiser et al. 2001, Mistri et al.
136 2004). For plots assigned to the low or high physical disturbance, an area of approximately 1 m²,
137 centring on but extending beyond the experimental plot was raked in a cross-hatched pattern. The
138 low treatment consisted of two strokes of the rake, perpendicular to one another. This level of
139 disturbance has previously been shown to illicit a response from intertidal communities
140 (Whomersley et al. 2010). The high treatment consisted of 6 strokes, each stroke perpendicular to
141 the previous. This disturbance was applied at the start of experiment and then monthly, over a
142 four-month period.

143 Nutrient plots were enriched using Scotts Osmocote Pro, 8-9 month coated fertiliser pellets (N:P:K
144 ratio of 16:4.8:8.3). These pellets which gradually release nutrients provide a controlled method of

testing the effects of chronic nutrient enrichment in soft sediment benthic environments without need for replenishment (Worm et al. 2000). Plots were given 0 g (zero), 500 g (low) or 1000 g (high) of fertiliser. The high nutrient treatment was based on the level of nutrient loading that might be experienced in the vicinity of the discharge point of a moderately sized sewage treatment plant (Morris & Keough 2002, 2003a, O'Brien et al. 2010) and the low treatment was set at half of this. For each of the plots assigned to the high or low nutrient treatment, nutrients were dispensed via five bags per plot, made from nylon panty-hose, among which the fertiliser was evenly distributed. These bags were spread evenly within each plot and were buried approximately 2–4 cm below the surface of the sediment. The nylon provided a fine permeable membrane through which nutrients could leach out into the plots. A procedural control tested for any experimental artefacts associated with burying the fertiliser; for this treatment nylon bags contained sediment in place of fertiliser and were deployed in the same manner as the nutrient treatments. Nylon bags containing slow release fertiliser or sediment (for the procedural control) were added to plots at the start of the experiment.

Sampling

Prior to initial manipulation of stressors, the chlorophyll and organic content of a subset ($n = 25$) of plots was sampled to determine background conditions at each site. Additionally, the spectral reflectance of the sediment and the macrofaunal community were sampled in each plot immediately prior to the manipulation of stressors. All variables were subsequently sampled monthly, for four months, in the case of sediment properties and at 2 (July) and 4 (September) months for macrofauna. Sampling of chlorophyll *a* content and spectral reflectance were together

166 used as proxies for the biomass of microphytobenthos (Tolhurst et al. 2005, Kromkamp et al.
167 2006). Sampling was conducted immediately prior to each re-application of the physical
168 disturbance treatment. Sampling at Lane Cove was not possible in the first month following initial
169 disturbance due to large amounts of overlying water caused by a sizable low pressure system. The
170 location of sample collection within each plot at each sampling time was noted, so as to avoid
171 repeated collection of samples from the same area.

172 The sediment organic content and chlorophyll *a* concentration was assessed through the contact
173 coring method (Ford & Honeywill 2002) whereby the top 2 mm of the sediment surface was flash-
174 frozen with liquid nitrogen and then freeze dried. A single randomly-positioned 2463 mm² core
175 was collected from each plot at each sampling time. Over a period of 48 hours, photosynthetic
176 pigments were extracted from a 200 mg subsample of sediment from each core using 1.5 ml of
177 90% acetone under dark conditions within a -80 °C freezer. Samples were agitated after 24 hours
178 for 10 seconds by using a vortex mixer. The chlorophyll *a* concentration of the acetone solution
179 was determined spectrophotometrically using the method of Jeffrey & Humphrey (1975) and the
180 chlorophyll *a* content of sediment was calculated per unit area (mg m⁻²). A second 2 g subsample
181 of sediment was taken from each contact core to determine the organic content through loss-on-
182 ignition (450 °C for 4 hours).

183 An Ocean Optics USB2000 spectroradiometer was used to measure the spectral reflectance of the
184 sediment surface *in situ*. Reflectance values (R) were made in the visible (675 nm) and infra-red
185 (750 nm) parts of the spectra, and used to calculate the Normalised Difference Vegetation Index

186 (NDVI), a measure of the photosynthetically-related biomass based upon reflectance of
187 chlorophyll *a* in the sediment (Kromkamp et al. 2006):

188
$$\text{NDVI} = (\text{R750} - \text{R675}) / (\text{R750} + \text{R675})$$

189 Three measurements were collected per plot allowing an average NDVI to be calculated.

190 Reflectance measurements were discarded where there was interference from surface water.

191 Nevertheless, at each sampling time measurements from at least $n = 3$ plots per treatment could
192 be included in the analyses.

193 One large core (10 cm diameter, 10 cm depth) was collected from each plot to quantify
194 macrofaunal biodiversity each of the sampling times indicated above. Sediment cores were taken
195 from between nylon bags, so as not to disrupt dispense of nutrients. Sediment core samples were
196 sieved through a 500 μm diameter mesh and material retained on the sieve was fixed in 10%
197 buffered formalin. Retained material was subsequently transferred into 70% ethanol and
198 examined under a dissecting microscope to separate fauna from other remaining material. Fauna
199 were identified to species level or morphospecies where this was not possible.

200 **Statistics**

201 Permutational analyses of variance (PERMANOVA: Anderson 2001, Anderson et al. 2008) were
202 used to analyse univariate and multivariate data. Although initially developed for multivariate
203 application, PERMANOVA can also be run on univariate data (Anderson et al. 2008) and has the
204 advantage over ANOVA that it does not have assumptions regarding the underlying distribution of
205 the data and can be used on any distance matrix (Anderson et al. 2008). Multivariate analyses,

206 using Bray Curtis dissimilarities, were run on macrofaunal composition data. Macrofaunal
207 composition data was square-root transformed prior to PERMANOVA in order to down-weight the
208 effect of species dominance and ordinated using two-dimensional non-metric multidimensional
209 scaling (nMDS). Univariate tests, using Euclidean distance matrices, were run on each of sediment
210 organic content, chlorophyll *a* content, NDVI, Shannon's diversity of macrofauna, total abundance
211 of macrofauna, species richness of macrofauna, as well as the abundance of macrofaunal species
212 that were key discriminators of multivariate differences among treatments.

213 First, for macrofauna and NDVI variables, three-way analyses on time 0 data, collected prior to
214 application of perturbations, and including the factors nutrient enrichment (fixed; 3 levels: zero
215 [0N], low [LN], high [HN]), physical disturbance (fixed; 3 levels: zero [0D], low [LD], high [HD]) and
216 site (fixed; 2 levels) tested for any pre-existing differences in variables of interest among plots and
217 between sites that coincided with their treatment assignment. Due to only a subset of contact
218 core samples taken in the first month we were unable to run a complete analysis testing
219 differences between treatments for chlorophyll *a* and organic content, however a one-way
220 analysis was conducted testing for differences between sites. Second, four-way analyses, with the
221 factors time (4 levels for most variables [1, 2, 3, 4 months after perturbation], and 2 levels for
222 macrofauna [2, 4 months]), site (2 levels, random), treatment (2 levels: undisturbed, disturbance
223 control) and plot (random; nested within site and treatment) tested for experimental artefacts of
224 the method of nutrient application. Third, five-factor analyses with the factors site (random, 2
225 levels); nutrient enrichment (fixed; 3 levels); physical disturbance (fixed; 3 levels); plot (random, 7
226 levels, nested within site x nutrients x disturbance) and time (random, 2 or 4 levels) tested for

227 interacting effects of the two stressors across both sites and all time periods. The inclusion of plot
228 as a nested factor enabled time to be treated as a repeated measure. Following these analyses
229 pairwise *post hoc* tests were conducted to identify significant differences between factors. Where
230 the number of possible permutations was lower than 100, Monte Carlo (MC) testing was run to
231 generate p-values from constructed asymptotic permutation distributions for the pseudo-*F*
232 statistic. All analyses were conducted using the PRIMER v6 statistical program with the
233 PERMANOVA+ addition (Clarke & Gorley 2006, Anderson et al. 2008).

234 The macrofaunal multivariate analysis revealed large spatial and temporal differences in
235 communities resulting in strong interactions between month and site. These prevented the
236 factors of interest (nutrient enrichment and physical disturbance) from being appropriately tested
237 and so separate tests were run for each month and site using a reduced model including only the
238 factors nutrient enrichment and physical disturbance. Key discriminating species, contributing to
239 multivariate differences in macrofaunal community structure, were identified by SIMPER analysis
240 and were defined as those having a dissimilarity/standard deviation ratio > 1 between nutrient or
241 physical disturbance treatments. Of these, species that were present at both sites were analysed
242 using the complete 5-factor model, with abundances 4th root transformed. Discriminating species
243 that were unique to a site were analysed under the reduced 2-factor model. Permutational
244 multivariate dispersions (PERMDISP) were analysed alongside the multivariate PERMANOVAs to
245 assess the extent to which any treatment effects were driven by differences in dispersion
246 between treatments and unless otherwise indicated were non-significant.

247 **Results**

248 **Microphytobenthic component**

249 Prior to experimental manipulations there were no differences in organic content or chlorophyll *a*
 250 content of sediments between the two sites, although NDVI was significantly greater at Lane Cove
 251 than Botany Bay (main effect of site PERMANOVA: $p < 0.05$; see supplemental materials). Across
 252 the two sites, there were no pre-existing differences in NDVI among plots that corresponded to
 253 treatment assignments (PERMANOVA $p > 0.05$, see supplemental materials; insufficient plots
 254 were sampled to address this question for organic content and chlorophyll *a*). At none of the
 255 sampling times following application of stressors were there experimental artefacts of the
 256 method of fertiliser burial on any of the three variables, organic content, chlorophyll *a* or NDVI
 257 (PERMANOVA $p > 0.05$; see supplemental material).

258 Organic content did not respond to the addition of stressors at either site (Table 1). By contrast,
 259 site-specific effects of stressors on the two measures of MPB biomass, chlorophyll *a*
 260 concentration and NDVI were observed (Table 1). Neither variable displayed interactive effects
 261 between the two stressors so additive effects could be interpreted. Chlorophyll *a* concentration
 262 displayed a significant interaction between physical disturbance, site and month (Table 1; Figures
 263 1A,B), whereas there was no effect of physical disturbance on NDVI at either site (Table 1). In
 264 Botany Bay (Figure 1A), although a slight negative impact of high physical disturbance on
 265 chlorophyll *a* was observed in August (month 3), prior to September (month 4) the effects of
 266 disturbance were non-significant (*a posteriori* pairwise comparisons $p > 0.05$; $0D = LD = HD$). In
 267 September, plots receiving the high physical disturbance treatment had a significantly lower

chlorophyll *a* concentration than those not receiving physical disturbance ($t = 1.99$, $p(\text{perm}) = 0.048$). There were no differences between the undisturbed and low disturbance treatments ($t = 1.59$, $p(\text{perm}) = 0.113$), or the low and high disturbance treatments ($t = 0.14$, $p(\text{perm}) = 0.898$). At Lane Cove (Figure 1B), lower levels of chlorophyll were observed in plots receiving high than low physical disturbance in July (month 2; $\text{HD} < \text{LD}$, $t = 3.42$, $p(\text{perm}) = 0.002$), but in August these two treatments did not significantly differ, and there were no significant differences between other pairs of treatments in either July or August (*a posteriori* pairwise comparisons: $p > 0.05$). In September, low disturbance resulted in significantly greater concentrations of chlorophyll *a* than in the undisturbed ($t = 2.34$, $p(\text{perm}) = 0.022$) or high disturbance treatment ($t = 2.97$, $p(\text{perm}) = 0.004$), but there was no difference between the high disturbance and undisturbed treatments ($t = 0.71$, $p(\text{perm}) = 0.497$) ($\text{LD} > \text{OD} = \text{HD}$).

Despite chlorophyll *a* displaying a significant interaction between nutrient addition and month (N x M interaction; Table 1) and a weak trend for a greater concentration of in plots receiving a low or high nutrient addition than no nutrients (Figure 1C, D), in none of the months were significant differences among nutrient treatments found (*a posteriori* pairwise comparisons: $p > 0.05$). By contrast, NDVI displayed a significant interaction between nutrient enrichment and site (Table 1), with effects of nutrient enrichment apparent in Botany Bay only (Figure 2). In Botany Bay, plots receiving either a low ($t = 2.16$, $p(\text{perm}) = 0.032$) or high nutrient addition ($t = 2.75$, $p(\text{perm}) = 0.011$) had a significantly greater NDVI than unenriched plots. There were no differences between the low and the high treatment ($t = 0.63$, $p(\text{perm}) = 0.557$) ($\text{ON} < \text{LN} = \text{HN}$).

288 **Macrofauna**

289 Analysis of macrofaunal community structure prior to manipulations revealed that multivariate
 290 community composition differed significantly between the sites (Pseudo- $F_{(1,108)} = 31.63$, $p(\text{perm})$
 291 $= 0.001$), with a greater abundance of invertebrates per plot at Lane Cove than Botany Bay
 292 (Pseudo- $F_{(1,108)} = 3.70$, $p(\text{perm}) = 0.031$). Sixty-five species were identified, of which 28 were
 293 common between the two sites, 19 species were unique to Botany Bay and 18 were unique to
 294 Lane Cove. Shannon Diversity and total plot species richness did not significantly differ between
 295 the two sites (PERMANOVA $p > 0.05$; see supplemental materials). At neither of the sites were
 296 there pre-existing differences among plots that corresponded to treatment assignments (see
 297 supplemental materials). Furthermore, at none of the sampling times were there experimental
 298 artefact associated of the method of nutrient addition on macrofauna (PERMANOVA $p > 0.05$; see
 299 supplemental materials).

300 In the analysis comparing stressor impacts to macrofauna between the two sites, and across all
 301 sampling times, there was no effects of nutrient enrichment or physical disturbance on
 302 community composition or biodiversity indices, however there was a significant interaction
 303 between month and site (PERMANOVA: $p(\text{perm}) < 0.05$). When sites and times were considered
 304 separately, stressor effects on macrofauna could not be distinguished in July (PERMANOVA, $p >$
 305 0.05) and in September, following four months of continued exposure to stressors, there was no
 306 interacting effect of nutrients and physical disturbance on any of the measures of macrofaunal
 307 community structure, at either site, allowing interpretation of main effects (Table 2). Whereas the
 308 September analysis revealed no effect of physical disturbance on any of the measures of

macrofaunal community structure, nutrient enrichment affected multivariate community composition at Botany Bay and at Lane Cove it had a weak, though non-significant effect on this variable (Table 2; Figure 3). At Botany Bay, the highly enriched treatment was significantly different to the unenriched treatment only ($t = 1.63$, $p(\text{perm}) = 0.031$), with no differences between the low and the highly enriched treatments ($t = 1.36$, $p(\text{perm}) = 0.077$) or between the low enrichment and unenriched treatments ($t = 1.04$, $p(\text{perm}) = 0.386$). Additionally at Botany Bay the multivariate dispersions were significantly greater in the enriched plots in comparison to the controls (PERMANOVA, *a posteriori* tests, $0N < LN = HN$; PERMDISP: $F_{(2,58)} = 8.94$, $p(\text{perm}) = 0.002$). There were no treatment effects on Shannon diversity for either Botany Bay or Lane Cove (Table 2). However, for Lane Cove, there was a treatment effect of nutrient enrichment on the total macrofaunal abundance whereby increasing enrichment caused a decrease in total abundance (Table 2; PERMANOVA, *a posteriori* tests, $0N > LN > HN$). Analysis of dispersions also revealed increased nutrient enrichment significantly decreased the dispersion of abundances at Lane Cove with no differences in dispersion of either low or highly enriched treatment ($0N > LN$, HN ; PERMDISP: $F_{(2,58)} = 7.06$, $p(\text{perm}) = 0.027$).

SIMPER analysis identified six discriminating species contributing most to dissimilarity between nutrient treatments in Botany Bay, four of which coincided with those found in Lane Cove. These were *Mysella* sp., *Mediomastus australiensis*, *Prionospio* sp., and *Nephtys australiensis*. These species were among the most abundant species found at each site. The additional discriminating species at Botany Bay were *Salinator fragilis* and *Owenia australis*. Lane Cove had a total of 12 discriminating species which also included two species of *Gammarus* amphipods, Oedicerotidae

330 sp. amphipods, *Scoloplos* sp., *Laternula* sp., *Platynereis uniseris*, *Australonereis ehlersi* and large
331 nematodes.

332 In repeated measures analyses that included both sites, *M. australiensis* did not display any
333 significant effects of nutrient enrichment or physical disturbance at either of the sites or at any of
334 the sampling times (Table 3). Nutrient enrichment caused a significant alteration of the
335 abundance of *Prionospio* sp. at Botany Bay only (nutrient enrichment x site interaction; Table 3; *a*
336 *posteriori* pairwise comparisons: $p < 0.05$; Figure 4). Pairwise comparisons revealed that at Botany
337 Bay, the high nutrient enrichment plots had significantly lower *Prionospio* sp. abundances than
338 the unenriched plots ($t = 2.42$, $p(\text{perm}) = 0.016$) whereas there were no significant differences
339 between the unenriched and low enrichment plots, or the low and high enrichment plots ($p >$
340 0.05).

341 Across both sites and all sampling times, there was a significant interaction between nutrient
342 enrichment and physical disturbance for the species *N. australiensis* (Table 3; Figure 4). There
343 were no significant differences due to the impact of disturbance among plots that received either
344 zero or high enrichment (*a posteriori* pairwise comparisons: $p > 0.05$). Among plots that received
345 low nutrient enrichment, abundances of *N. australiensis* were significantly greater in plots that
346 also received low physical disturbance than those that were not disturbed ($LD > 0D$; $t = 16.64$,
347 $p(\text{perm}) = 0.013$), with no significant difference between plots receiving high or low physical
348 disturbance ($t = 0.40$, $p(\text{perm}) = 0.829$) and between plots receiving high or no physical
349 disturbance ($t = 1.94$, $p(\text{perm}) = 0.223$). By contrast, among plots receiving high or zero nutrient
350 enrichment, there was no significant effect of physical disturbance on *N. australiensis* abundance

351 (*a posteriori* pairwise comparisons: $p > 0.05$). When the interaction was examined around the
 352 other way around, there was no significant effect of nutrient enrichment, within any of the three
 353 levels of physical disturbance (*a posteriori* pairwise comparisons: $p > 0.05$).

354 *Mysella* sp. displayed a three-way interaction between nutrient enrichment, physical disturbance
 355 and month, with no differences between sites (Table 3; Figure 4). Among unenriched plots, there
 356 was no significant effect of physical disturbance in either of the months (*a posteriori* pairwise
 357 comparisons: $p > 0.05$). Among plots subjected to low nutrient enrichment, abundances were
 358 greater in plots receiving high than no physical disturbance in each of the months (HD > OD; July: t
 359 = 21.30, $p(\text{MC}) = 0.037$; September: $t = 19.32$, $p(\text{MC}) = 0.031$) and in July, there was also a
 360 significant difference between the high and low disturbance treatment (HD > LD; $t = 22.42$, $p(\text{MC})$
 361 = 0.037) but all other pairwise comparisons were non-significant ($p > 0.05$). Among plots receiving
 362 high nutrient enrichment, plots receiving high physical disturbance had greater abundances of
 363 *Mysella* sp. than undisturbed plots in July (HD > OD; $t = 31.87$, $p(\text{MC}) = 0.017$), whereas in
 364 September, highly disturbed plots contained fewer individuals than those that were undisturbed
 365 (HD < OD; $t = 12.33$, $p(\text{MC}) = 0.049$), with no other pairwise comparisons between levels of
 366 physical disturbance significant for either of the sampling times ($p > 0.05$). Examining the
 367 interaction around the other way, in July, plots receiving no physical disturbance had lower
 368 abundances of *Mysella* sp. when they received low as compared to no nutrient enrichment (ON >
 369 LN; $t = 51.61$, $p(\text{MC}) = 0.013$), but other pairwise contrasts revealed no other significant
 370 differences (*a posteriori* pairwise comparisons: $p > 0.05$). In September, both the low and high
 371 nutrient treatments had lower abundances of *Mysella* sp. in comparison to the unenriched

372 treatment (low: $t = 17.51$, $p(\text{MC}) = 0.33$; high: $t = 41.83$, $p(\text{MC}) = 0.017$) but did not significantly
 373 differ from one another ($0\text{N} > \text{LN} = \text{HN}$; $t = 0.04$, $p(\text{MC}) = 0.977$). There was no effect of nutrient
 374 enrichment on *Mysella* sp. among plots receiving low or high physical disturbance (*a posteriori*
 375 pairwise comparisons: $p > 0.05$).

376 Few of the species that were key discriminating taxa at only one of the two sites displayed
 377 significant responses to stressors (see supplemental materials: Table S4). At Lane Cove, *Scoloplos*
 378 sp. displayed a significant interaction between nutrient enrichment and physical disturbance
 379 ($\text{Pseudo-}F_{(2,52)} = 2.91$, $p(\text{perm}) = 0.039$; Figure 5A). Among plots subjected to zero physical
 380 disturbance, plots receiving low ($t = 2.23$, $p(\text{perm}) = 0.034$) and high ($t = 2.13$, $p(\text{perm}) = 0.050$)
 381 nutrient contained fewer *Scoloplos* sp. than those receiving zero enrichment, with no differences
 382 between the low and high nutrient treatments ($0\text{N} > \text{LN} = \text{HN}$). By contrast, among plots receiving
 383 low or high physical disturbance, there was no effect of nutrient enrichment (PERMANOVA *a*
 384 *posteriori* tests: $p > 0.05$). When the interaction was examined around the other way, among plots
 385 subjected to zero nutrient enrichment, high physical disturbance resulted in significantly fewer
 386 *Scoloplos* sp. as compared to undisturbed controls ($0\text{D} > \text{HD}$; $t = 2.46$, $p(\text{perm}) = 0.030$) whereas
 387 there were no differences between plots receiving low and no disturbance ($t = 2.38$, $p(\text{perm}) =$
 388 0.064) or low and high disturbance ($t = 0.12$, $p(\text{perm}) = 0.99$). Among plots receiving low or high
 389 nutrient enrichment, there was no significant effect of disturbance on *Scoloplos* sp. (PERMANOVA
 390 *a posteriori* tests: $p > 0.05$). At Lane Cove, nematodes displayed a significant main effect of
 391 nutrient enrichment only ($\text{Pseudo-}F_{(2,52)} = 3.25$, $p(\text{perm}) = 0.045$; Figure 5B) whereby abundance

392 decreased with increasing nutrient enrichment (ON > LN > HN; PERMANOVA *a posteriori* tests: $p <$
393 0.05).

394 **Discussion**

395 The limited number of mesocosm studies focussing on the interaction between physical
396 disturbance and nutrient enrichment have highlighted the potential for interactions between
397 these two stressors (Widdicombe & Austen 2001, Austen & Widdicombe 2006). This study sought
398 to expand upon this earlier work by examining the interaction between nutrient enrichment and
399 physical disturbance, under larger-scale field conditions that offer an element of ecological
400 realism To our knowledge, it represented the first field-based study to examine the multiple
401 effects of these two stressors in an intertidal sedimentary habitat (but see Rossi & Underwood
402 2002 for a discussion on the impacts of organic matter burial that result both from physical
403 disturbance of sediments and nutrient enrichment during decay). It found site-specific effects of
404 nutrient enrichment and physical disturbance, with limited interactions between stressors. The
405 study thereby demonstrates the role environmental context plays on mediating the impact of
406 multiple stressors, and raises the possibility that communities may be more resilient to increased
407 stress than can be predicted from laboratory studies alone.

408 Of the two stressors examined by this study, nutrient enrichment generally elicited stronger
409 ecological responses than physical disturbance. Eastern Australian estuaries are, in general,
410 oligotrophic and phosphorus-limited (Bishop et al. 2006, Scanes et al. 2007, Kelaher et al. 2013).
411 In nutrient-limited systems such as these, nutrient enrichment typically stimulates bottom-up
412 responses of the MPB (O'Brien et al. 2010, Pascal et al. 2013). This growth may then lead to

413 cascading positive effects on higher trophic levels (York et al. 2012). Nevertheless, because excess
414 primary production can also result in deterioration of sediment conditions through over-
415 stimulation of oxygen-consuming bacteria, the effects of nutrient enrichment on macrofaunal
416 abundance and richness can range from positive (Morris & Keough 2003a, b) to negative (Fitch &
417 Crowe 2010, Botter-Carvalho et al. 2014), depending on the level of enrichment and
418 environmental conditions (Pearson & Rosenberg 1978).

419 In the present study, an effect of nutrient enrichment on MPB was identified at both study sites
420 (although effects were stronger in Botany Bay), and the effect was positive, in agreement with
421 mesocosm studies (e.g. Sundback et al. 2010). While nutrient additions acted to alter macrofaunal
422 community structure at each of the sites, effects were weaker than expected, with few species
423 significantly affected by stressors, and the nature of effects variable between these. It has been
424 hypothesised that estuarine communities have an inherent ability to resist stress at levels that
425 would push other aquatic systems over a threshold, and which are above and beyond that which
426 they are subjected to daily (e.g. salinity fluctuations, tides, and emersion periods; see Elliott &
427 Whitfield 2011). Within nature, biotic interactions may reduce the impacts of nutrient
428 enrichment. The limited effects of nutrient enrichment observed in this study indicate there could
429 be a mix of top down and bottom up effects impacting the community. Although in nutrient-
430 limited systems, nutrient enrichment facilitates autotrophic growth, this effect can become
431 masked by top down processes where increased productivity is matched by increased grazing
432 pressure (Pascal et al. 2013). Alternatively, the weak effects of nutrient enrichment observed in
433 the present study may be a function of other abiotic and biotic characteristics of the sites, an

434 aspect that would be controlled for in closed systems. Sedimentary grain size and starting
435 community composition can play an important role in interpreting how communities will respond
436 to stressors (Lindegarth & Hoskin 2001, Rossi & Underwood, 2002, Whomersley et al. 2010). The
437 relatively weak effects of nutrient enrichment detected in this study are consistent with larger-
438 scale surveys that indicate that nutrient enrichment is potentially a weak driver of change in
439 benthic community composition in comparison to environmental characteristics, such as grain
440 size (Nicastro & Bishop 2013). Alternatively, the weak effects of nutrient enrichment seen in this
441 study may reflect the small scale of manipulations. Larger-scale disturbances alter system
442 dynamics ultimately impacting recovery and community resilience (Ellis et al. 2000).

443 Both MPB and macrofauna displayed a limited to neutral response to physical disturbance despite
444 predictions, based on previous field studies, of negative effects of this stressor from destruction
445 of sediment structure, burrows and damage to softer-bodied species (Hall 1994, Brown & Wilson
446 1997, Hall & Harding 1997, Dernie et al. 2003, Rossi et al. 2007). Community or species-specific
447 responses to physical disturbance have been previously noted to vary according to environmental
448 context (Whomersley et al. 2010) and previous mesocosm experiments have found that, in line
449 with predictions of the Dynamic Equilibrium Hypothesis (Huston 1979), the effects of physical
450 disturbance vary across productivity gradients caused by nutrient enrichment (Widdicombe &
451 Austen 2001, Austen & Widdicombe 2006). Consistent with the Dynamic Equilibrium Hypothesis,
452 a number of macrofaunal species in this study displayed responses to physical disturbance that
453 varied according to the prevailing levels of nutrient enrichment. Overall, however, the number of

454 species exhibiting such a non-additive responses was few, and nutrient enrichment was the
455 dominant stressor impacting community composition in these systems.

456 While small scale disturbances such as raking and trampling sediments have been shown to
457 negatively impact sediment communities in other studies (Rossi et al 2007; Whomersley et al
458 2010), the spatial scale and frequency of raking may simply not have been great enough to elicit a
459 strong response in the communities examined here. In this experiment, plots were less than 1 m
460 in diameter, allowing species to rapidly recolonise from outside affected areas (see Lee et al.
461 2011). This small scale of physical disturbance might be analogous to that caused by clam raking,
462 by boat-run up on intertidal sediments or by propeller scaring. However, for disturbances, such as
463 storms, that cause physical disturbance at the scale of the entire site, movement of species
464 between affected areas would not be possible. Treatment effects at this level are likely to
465 facilitate the selection for more resilient organisms that are able to tolerate stressors (Sanford &
466 Kelly 2011). The relatively (low) monthly frequency of raking may also have contributed to the
467 weak to absent responses of taxa to physical disturbance. The frequency of disturbance is an
468 important factor determining the magnitude of impact (Connell 1978, White & Pickett 1985), with
469 infrequent disturbances providing opportunity for organisms to recolonise in between,
470 particularly if the spatial scale of the disturbance is small, but frequent disturbances preventing
471 recovery before the next perturbation. Nevertheless, in Northern Europe, a single raking event
472 covering an area of 36 m², that perturbed large benthic species, had ecological impacts that
473 extended a year (Kaiser et al. 2001). Impacts are, therefore, likely to be dependent on the size and
474 scale of the raking event, as well as the species present. Although the physical disturbance

475 imposed by repeatedly sampling of sediments may have complicated results by leading to small
476 scale disturbance across all experimental plots, irrespective of disturbance treatment (Lindegarth
477 and Underwood 2000), we were careful to sample different areas on each sampling date.

478 Within the literature, there are many examples of context dependent effects of manipulations,
479 even across geographically proximate and/or environmentally similar locations (e.g Whomersley
480 et al. 2010, Bishop & Kelaher 2013, Gladstone-Gallagher et al. 2014). In this study, the differing
481 response between the two sites to environmental perturbations may be a direct effect of
482 environmental differences between sites, or an indirect effect arising from differences in their
483 community composition. Although the two sites did not differ in background sediment organic
484 content or chlorophyll concentration and were selected to be of similar grain size, other
485 environmental variables are likely to have differed. Botany Bay is a large industrial area with a
486 history of contamination whereas Lane Cove is surrounded by bushland and is used for
487 recreational purposes such as bushwalking, boating and fishing. Habitat characteristics such as
488 grain size, organic content and water content mediate the speed of benthic recovery from
489 perturbations (Dernie et al. 2003). Additionally, under natural conditions, habitat heterogeneity
490 can buffer against treatment effects (Godbold et al. 2011) or lead to substantial background
491 variation, against which treatment effects are hard to detect (Bulling et al. 2008). Community
492 structure may mediate stressor impacts by dictating the functional capability of the ecosystem,
493 and the ways in which species may interact to buffer the effects of stressors (Bulling et al. 2008,
494 Godbold & Solan, 2009).

495 Additionally, differences between the sites in background stressors may have contributed to the
496 differing sensitivity of their communities to the experimental perturbations. Background stress
497 can mediate how a system will respond to further stress via two main mechanisms. First,
498 prevailing stress may select for organisms that have enhanced resistance to local stressors and so
499 are able to withstand further disturbance (Sanford & Kelly, 2011). Second, prevailing stressors can
500 determine how far away a system is from a tipping-point beyond which the application of
501 additional stress pushes the system over a threshold value, resulting in a system collapse (Pearson
502 & Rosenberg, 1978; Whomersley et al., 2010). How far away systems are from tipping points is
503 related to the conditions to which fauna are adapted and the trade-offs they have made either
504 through evolutionary or local adaptations (Sanford & Kelly 2011, Botero et al. 2015). In dynamic
505 environments subjected to multiple stressors such as estuaries, it would be expected that
506 communities are dominated by species that are resilient to perturbation (Sanford & Kelly 2011).
507 Only where stressors exceed the threshold of resilience is there system collapse or change to
508 another stable state.

509 Although previous mesocosm studies have identified interactions between nutrient enrichment
510 and physical disturbance (e.g. Widdicombe & Austen 2001), our study demonstrates that the
511 results of such studies cannot necessarily be used to infer responses of benthic communities to
512 stressors in the field. While some studies have found agreement between the outcomes of field
513 and mesocosm experiments (e.g. Sundback et al. 2010), mesocosm studies can never completely
514 replicate the natural environment and conclusions about the way in which multiple stressors
515 interact can differ between these approaches (Przelawski et al. 2005; Crain et al. 2008, Alsterberg

et al. 2014). For example, Cowie et al (2000) observed a negative effect of physical disturbance on the macrofauna in both experimental mesocosms and *in situ* treatments; however, the strength of the decline of species was greater in mesocosm experiments. Adverse effects are likely to be amplified in mesocosm studies due to the stress imposed on organisms as a consequence of handling and containment in confined conditions, and because processes such as recruitment that act to weaken impacts in field scenarios cannot always occur (see Cowie et al. 2000). The heterogeneity of environments such as mudflats and the patchiness of species can contribute to the differing responses observed between laboratory and mesocosm studies (Crain et al. 2008), or even between different field sites (Norkko et al. 2010). While mesocosms provide a controlled environment, there is a growing evidence that environmental context (such as prevailing nutrient status) are often the determinant factors governing how systems will respond to stress (Sundback 2007; Bishop & Kelaher 2013; O'Connor & Donohue, 2013). Thus it follows that while mesocosm and laboratory experiments are often a quick and simple tool in understanding the consequences of change, these experiments should be utilised to formulate theories to be tested empirically (see Benton et al. 2007), field experiments must be used to determine true impacts of stressors under naturally occurring conditions. Furthermore, the implications of multiple stressor impacts are well known to vary depending upon the experimental protocol (laboratory/mesocosm; see Crain et al. 2008 and references therein) or the responses measured (Alsterberg et al. 2014). The differing method of nutrient enrichment between our study, which applied fertiliser to plots, and the previous mesocosm studies that enriched sediments via application of dried and ground-up *Ascophyllum* (Widdicombe & Austen 2001, Austen & Widdicombe 2006), may have contributed to differences in the conclusions of these. Similarly, the differing frequency of physical disturbance

between our study, which raked plots monthly, and the mesocosm experiments, that raked plots daily to monthly (Widdicombe & Austen 2001, Austen & Widdicombe 2006) may also have led to differences.

Conclusions

This study analysed the combined impact of selected multiple stressors –nutrient enrichment and physical disturbance – in a field setting. While both stressors have been previously identified to be detrimental to communities, and in mesocosm experiments produce non-additive effects, the combined impacts observed in this study were weak, mainly additive (at least at the chosen levels of stress), and mediated by environmental context. The differing results of this *in situ* study to those of previous mesocosm experiments, and the site-specificity of effects, highlight the important role that abiotic and biotic features of the environment play in mediating stressor effects. Furthermore, the resilience of benthic communities will determine their response to multiple stressors. The minor responses observed within this study highlight that benthic sedimentary communities are more resilient to increased stress than previous studies would indicate. Studies are now needed that examine which aspects of the environment or community are most important in mediating stressor impacts.

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Table 1: PERMANOVA analyses examining the interacting effects of Nutrients (N), Disturbance (D), Month (M), and Site (S) on organic content, chlorophyll α concentration and Normalised Difference Vegetation Index (NDVI) in Botany Bay and Lane Cove estuaries. Plot (P) was nested within Nx Dx S. Nutrient enrichment and physical disturbance factors contained 3 levels of applied stress (Zero, Low and High). The three months (2 – July, 3 – August, 4 – September) during which both sites were sampled are included in the analysis. n=3-7 for all treatments levels

Factor	Organic content			Chlorophyll α			NDVI		
	Df	Pseudo-F	P(perm)	df	Pseudo-F	P(perm)	df	Pseudo-F	P(perm)
N	2	1.68	0.255	2	3.95	0.070	2	1.22	0.406
D	2	3.47	0.076	2	2.49	0.143	2	0.34	0.916
M	2	1.60	0.371	2	0.84	0.492	3	0.58	0.693
S	1	7.62	0.002	1	0.12	0.747	1	2.59	0.116
NxD	4	0.54	0.859	4	1.10	0.422	4	0.75	0.696
NxM	4	0.42	0.821	4	27.40	0.002	6	1.45	0.336
NxS	2	0.54	0.828	2	1.25	0.282	2	3.42	0.039
DxM	4	0.58	0.730	4	0.08	0.981	6	0.79	0.615
DxS	2	0.42	0.916	2	1.11	0.323	2	2.33	0.104
MxS	2	2.37	0.078	2	9.88	0.001	3	25.91	0.001
NxDxM	8	1.24	0.363	8	1.80	0.221	12	0.53	0.855
NxDxS	4	1.46	0.166	4	0.47	0.945	4	1.23	0.262
NxMxS	4	0.78	0.572	4	0.05	0.998	6	1.54	0.161
DxMxS	4	2.10	0.054	4	3.05	0.015	6	1.55	0.174
P(NxDxS)	107	1.83	0.002	112	1.56	0.005	110	1.93	0.001
NxDxMxS	8	1.30	0.230	8	0.83	0.577	12	1.69	0.083
Res	179			187			301		

Key: **Bold** = significant effect at $p < 0.05$

Table 2: Two-way PERMANOVA analyses examining the interacting effects of Nutrients (N) and Disturbance (D) on the macrofaunal community at sites within Botany Bay and Lane Cove estuaries. Nutrient enrichment and physical disturbance factors contained 3 levels of applied stress (zero, low or high) over 4 months (M). n=5-7 for all treatments levels

Species richness				Abundance		Shannon Diversity		Multivariate	
	df	Pseudo-F	p(perm)	Pseudo-F	p(perm)	Pseudo-F	p(perm)	Pseudo-F	p(perm)
Botany Bay:									
N	2	0.67	0.562	0.51	0.618	1.20	0.311	1.92	0.035
D	2	0.36	0.702	0.19	0.846	0.64	0.523	1.31	0.181
NXD	4	0.48	0.767	0.52	0.766	0.55	0.699	0.98	0.476
Res	52								
Lane Cove:									
N	2	1.22	0.270	3.66	0.027	0.47	0.604	1.48	0.059
D	2	0.34	0.727	0.70	0.493	0.59	0.548	0.68	0.882
NXD	4	0.46	0.750	0.50	0.714	1.91	0.108	0.85	0.863
Res	52								

Key: **Bold** = significant effect at $p < 0.05$

Table 3: PERMANOVA analysis examining the interacting effects of Nutrients (N), Disturbance (D), Month (M), and Site (S) on the key discriminating species common to both sites within Botany Bay and Lane Cove estuaries. Plot (P) was nested within Nx Dx S. Nutrient enrichment and physical disturbance factors contained 3 levels of applied stress (zero, low or high), with sampling after 2 and 4 months (i.e. July, September). n=5-7 for all treatment levels

Factor	df	<i>Mediomastus australiensis</i>		<i>Prionospio</i> sp.		<i>Nephtys australiensis</i>		<i>Mysella</i> sp.	
		Pseudo-F	P(perm)	Pseudo-	P(perm)	Pseudo-F	P(perm)	Pseudo-	P(perm)
N	2	0.69	0.629	0.56	0.697	0.46	0.758	1.17	0.509
D	2	0.78	0.586	0.66	0.631	1.62	0.324	0.17	0.949
M	1	4.22	0.326	0.96	0.629	0.11	0.506	35.18	0.343
S	1	13.98	0.001	2.90	0.086	14.03	0.001	7.15	0.010
NxD	4	0.21	0.977	1.87	0.197	4.66	0.026	2.87	0.087
NxM	2	1.04	0.497	8.67	0.104	1.90	0.355	483.73	0.003
NxS	2	0.68	0.530	2.64	0.045	2.13	0.138	1.15	0.310
DxM	2	2.73	0.264	1.45	0.375	0.79	0.565	6.32	0.132
DxS	2	0.80	0.466	1.11	0.397	0.48	0.637	0.69	0.512
MxS	1	4.73	0.037	8.06	0.007	11.36	0.002	0.58	0.455
NxDxM	4	4.22	0.092	0.92	0.516	0.27	0.893	6.81	0.045
NxDxS	4	0.92	0.544	0.81	0.681	0.68	0.772	0.60	0.867
NxMxS	2	1.69	0.193	0.25	0.769	0.20	0.833	0.01	0.989
DxMxS	2	0.47	0.639	0.89	0.429	1.25	0.299	0.07	0.933
P(NxDxS)	11	1.46	0.033	1.12	0.295	0.97	0.570	3.08	0.001
NxDxMxS	4	0.79	0.538	1.30	0.290	0.74	0.564	0.10	0.983
Res	10								

Key: **Bold** = significant effect at $p < 0.05$

763 Figure 1: Mean (\pm SE) chlorophyll *a* concentration (mg m^{-2}) of surface sediments in
764 experimental plots. Differences among disturbance treatments through time at Botany Bay
765 (A) and Lane Cove (B). Differences among nutrient treatments through time at Botany Bay (C)
766 and Lane Cove (D). Sampling within Lane Cove could not be done in June due to large
767 amounts of overlying water. For all graphs, 0 treatment = circle, solid line; Low treatment =
768 square, long dashed line; High treatment = triangle; short dashed line. $n = 3-7$ for all
769 treatment levels.

770 Figure 2: Mean (\pm SE) normalised difference vegetation index (NDVI) at sites in Botany Bay (BB)
771 and Lane Cove (LC) following 4 months of continued nutrient enrichment and physical
772 disturbance crossed at 3 levels (0, L –Low, and H – High). Data averaged across all months
773 and physical disturbance treatments as there was no month \times nutrient or disturbance \times
774 nutrient enrichment interaction. $n = 3-7$ for all treatment levels. Letters indicate statistically
775 significant differences between treatments (PERMANOVA post hoc tests).

776 Figure 3: Two-dimensional nMDS configuration displaying macrofaunal composition at Botany Bay
777 (A) and Lane Cove (B) following 4 months of zero (0) low (L) and high (H) nutrient enrichment

(N) and physical disturbance (D). Points represent individual plots. Data square root transformed and matrix based upon Bray Curtis similarity. N = 5-7

Figure 4: Mean (+/- SE) abundance of common macrofaunal species in experimental plots at Botany Bay and Lane Cove following 4 months of nutrient enrichment and physical disturbance crossed at 3 levels (0, L – Low, and H – High) each. N = 5-7.

Figure 5: Mean (+/- SE) abundance of A) *Scoloplos* sp. and B) nematodes in experimental plots at Lane Cove following 4 months of nutrient enrichment and physical disturbance crossed at 3 levels (0, L –Low, and H – High) of each factor. N = 5-7

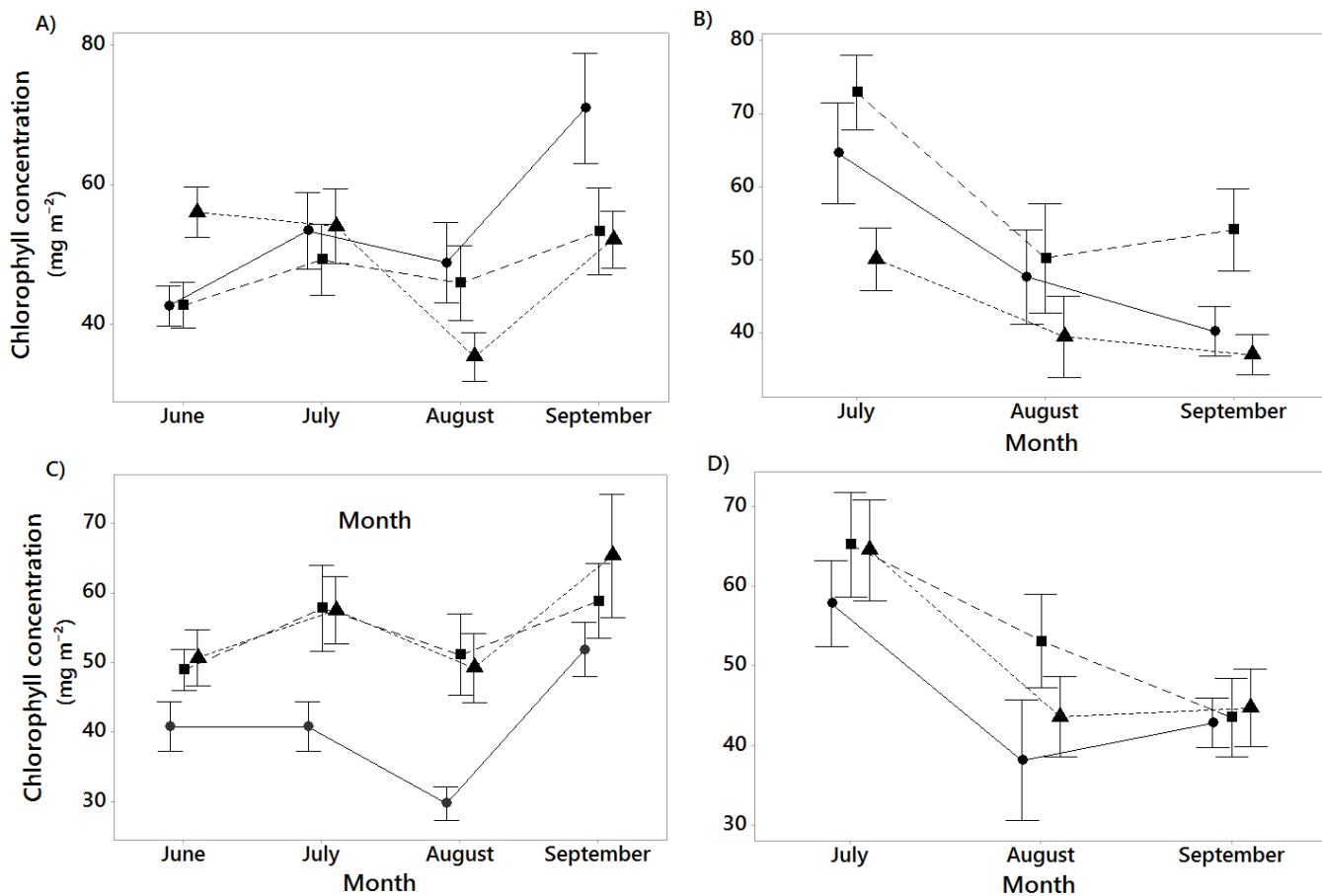


Figure 1:

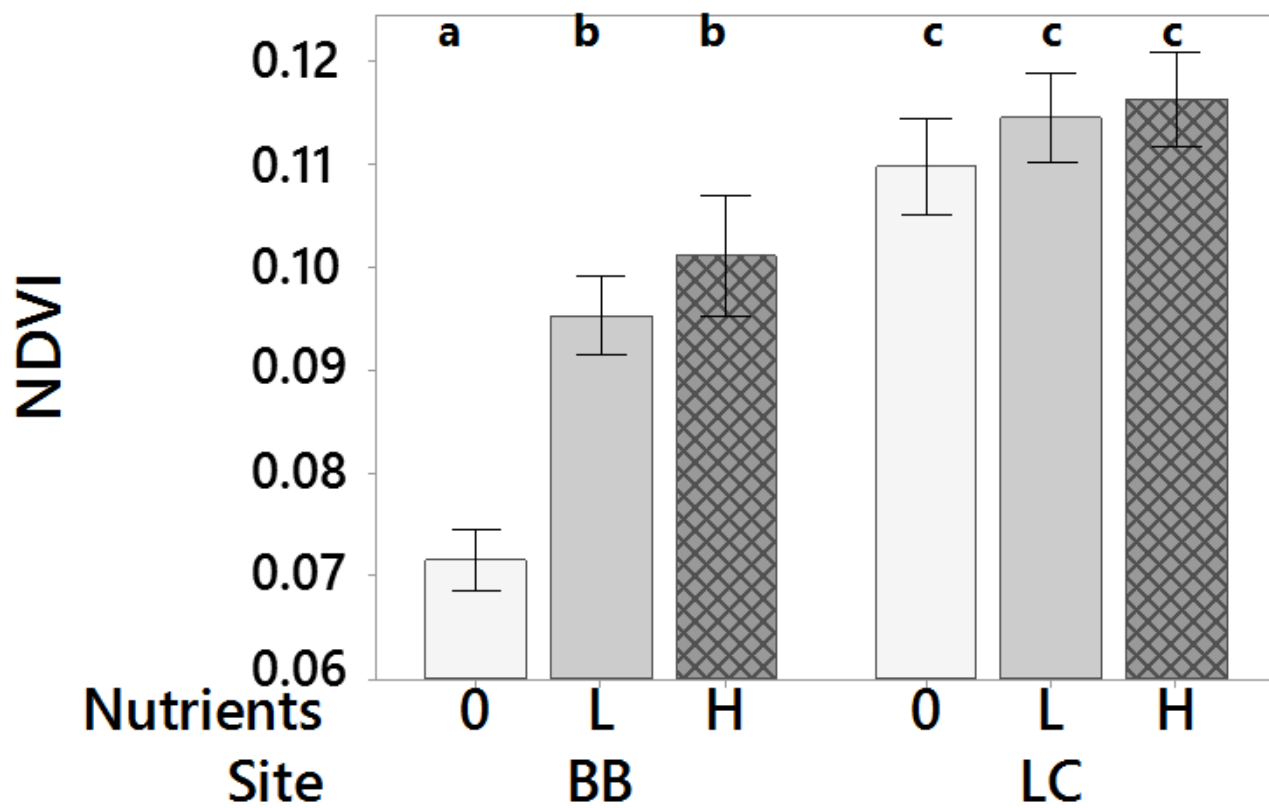


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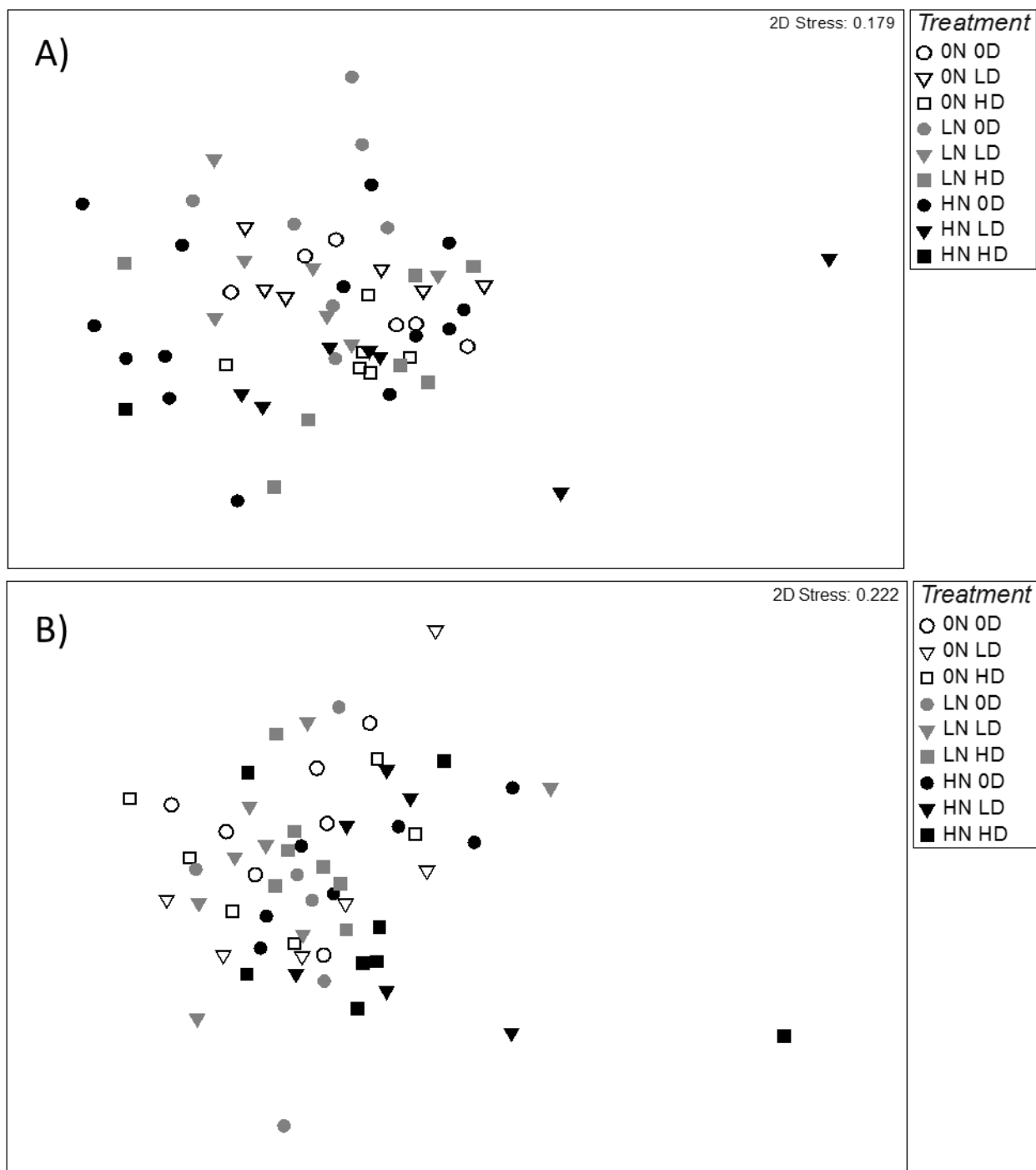


Figure 3:

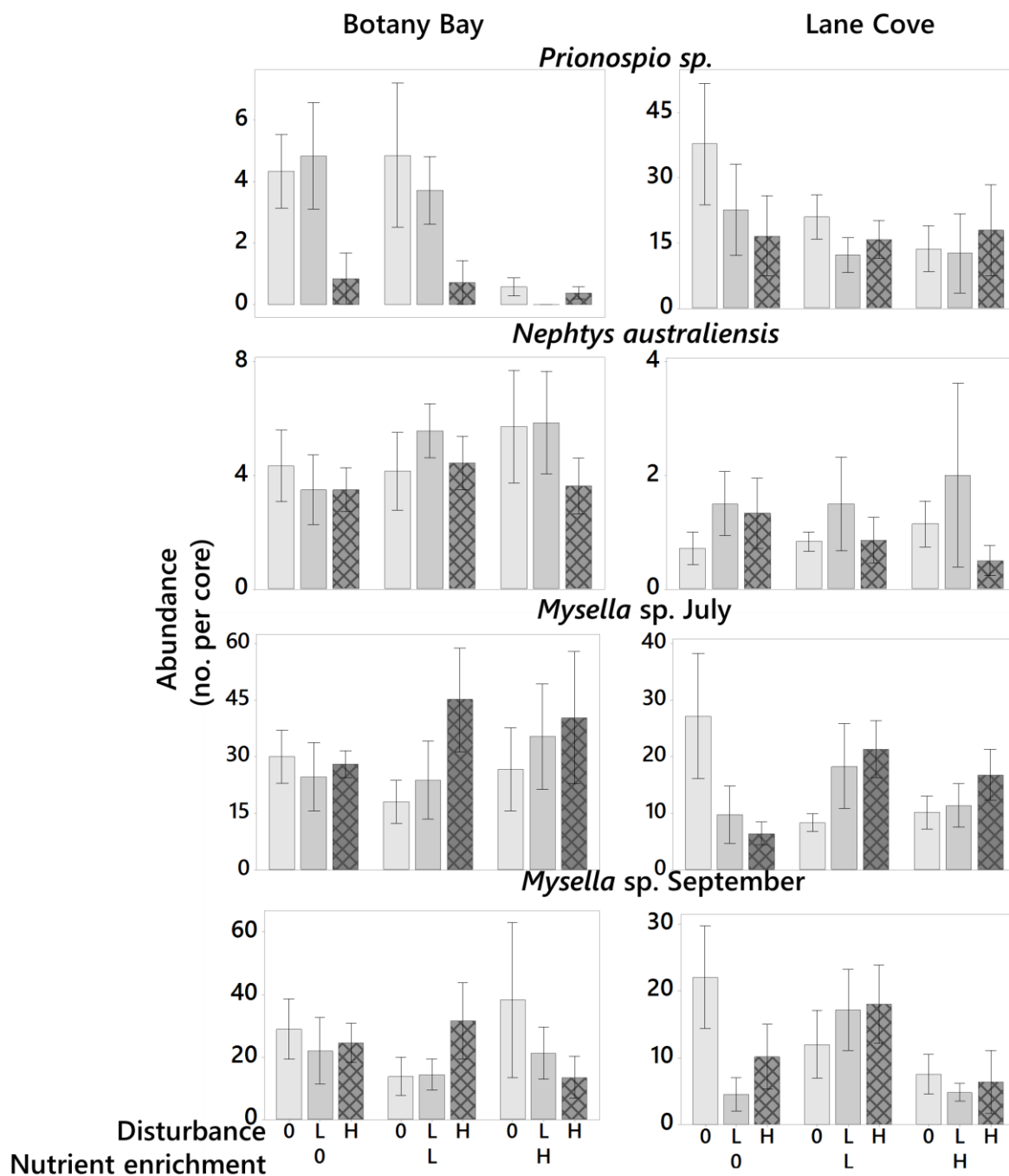


Figure 4:

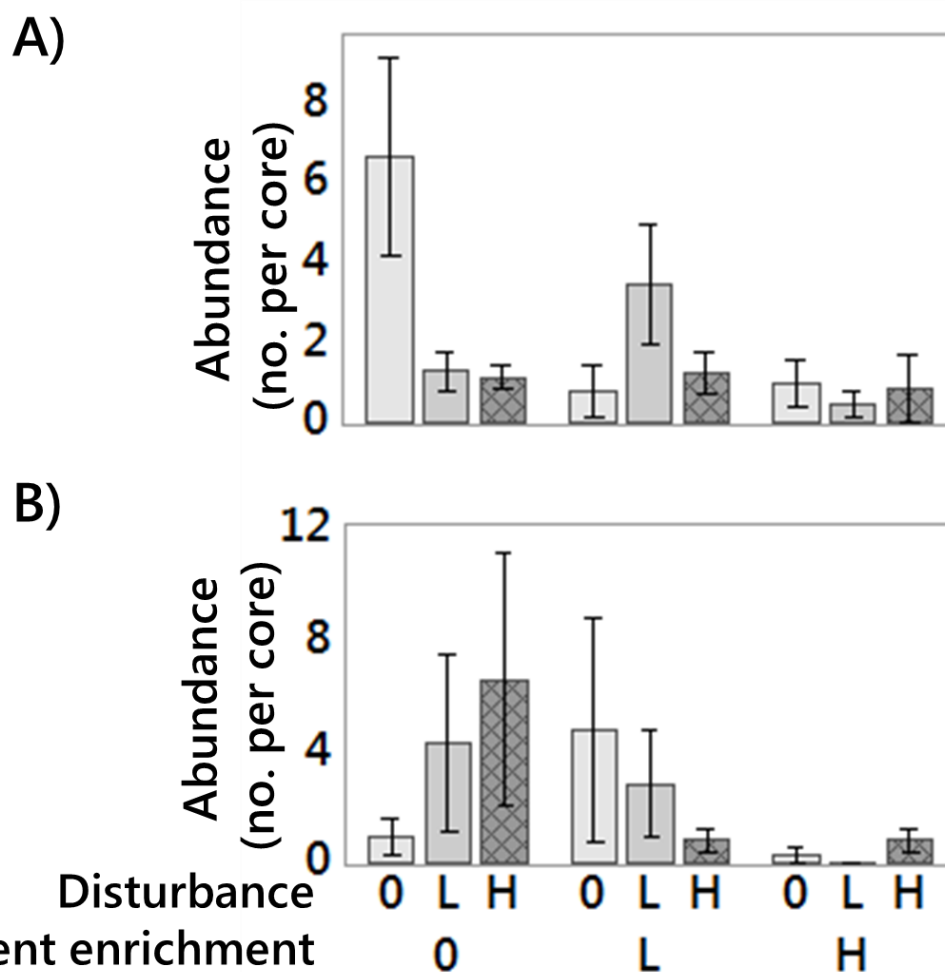


Figure 5: